

CLAIMS

1. A method for the *in vitro* translation of a ribonucleic acid template, said ribonucleic acid having both a 5' cap and a 3' poly A tail, said method comprising incubating a cell  
5 extract of a multicellular eukaryote with said ribonucleic acid template under conditions such that translation of the RNA template to produce its encoded protein by one or more components in the cell extract occurs and the amount of the encoded protein thus produced is greater than the total of (a) the amount of the encoded protein that is produced under said conditions when the ribonucleic acid template has a 5' cap but no  
0 3' poly A tail, plus (b) the amount of the encoded protein that is produced under said conditions when the ribonucleic acid template has a 3' poly A tail but no 5' cap.
2. The method according to claim 1 wherein the cell extract is an animal cell extract.
- 5 3. The method according to claim 2 wherein the cell extract is a mammalian cell extract.
4. The method according to claim 3 wherein the cell extract is a human cell extract.
5. The method according to claim 2 wherein the cell extract is an insect cell extract.
- 0 6. The method according to claim 5 wherein the cell extract is a *Drosophila* cell extract.
7. The method according to claim 6 wherein the cell extract is a *Drosophila* embryo cell extract.
- 5 8. The method according to claim 7 wherein the *Drosophila* embryo cell extract is prepared by a method comprising dechorionating *Drosophila* embryos in an aqueous isotonic buffer comprising detergent and bleach.
- 0 9. The method according to claim 8 wherein the detergent is Triton X-100 and the bleach is sodium hypochlorite.

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10. The method according to either of claims 8 or 9 which further comprises after dechorionating, the steps of washing the embryos; homogenizing the embryos to produce a homogenate; centrifuging the homogenate; and recovering non-pelleted material from the centrifuged homogenate.
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11. The method according to claim 6 wherein the cell extract is a *Drosophila* ovary cell extract.
12. The method according to claim 11 wherein the *Drosophila* ovary cell extract is prepared
- 0 by a method comprising (a) obtaining *Drosophila* ovaries in isotonic medium; (b) washing the ovaries so as to transfer the ovaries slowly from isotonic medium to hypotonic medium, such that the volume of the ovaries increases; (c) homogenising the ovaries to produce a homogenate; (d) centrifuging the homogenate to form an ovary cell extract; and (e) recovering non-pelleted material from the centrifuged homogenate.
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13. The method according to any one of the preceding claims wherein said conditions comprise the presence of the following: creatine phosphate, creatine kinase, potassium and magnesium salts, spermidine, amino acids, a reducing agent, and tRNA.
- 0 14. The method according to any one of the preceding claims wherein said conditions comprise a temperature in the range of 18° to 37°C, and said incubating is for at least 90 minutes.
15. The method according to any one of the preceding claims wherein said incubating is at
- 5 25°C.
16. A method for the preparation of a *Drosophila* embryo extract comprising:
- a) dechorionating *Drosophila* embryos in an aqueous isotonic buffer comprising detergent and bleach;
- 0 b) washing the embryos;
- c) homogenizing the embryos to produce a homogenate;
- d) centrifuging the homogenate; and
- e) recovering non-pelleted material from the centrifuged homogenate.

17. A method for the preparation of a *Drosophila* ovary cell extract comprising (a) obtaining *Drosophila* ovaries in isotonic medium comprising detergent and bleach; (b) washing the ovaries so as to transfer the ovaries slowly from isotonic medium to hypotonic medium, such that the volume of the ovaries increases; (c) homogenising the ovaries to produce a homogenate; (d) centrifuging the homogenate to form an ovary cell extract; and (e) recovering non-pelleted material from the centrifuged homogenate.
18. A method according to either of claims 16 or 17, wherein said detergent is non-ionic.
19. A method according to any one of claims 16-18, wherein said homogenising is performed in the presence of protease inhibitors.
20. A method according to any one of claims 16-19 wherein said buffer is EW buffer (0.7% NaCl, 0.4% Triton X-100).
21. A method according to any one of claims 16-20, wherein said embryos or ovaries are incubated in said isotonic buffer for between 1 and 9 minutes.
22. A method according to claim 21, wherein said embryos or ovaries are incubated for about 3 minutes.
23. A method according to any one of claims 16-22, wherein said embryos or ovaries are incubated at between 18 and 37°C.
24. A method according to claim 23, wherein said embryos or ovaries are incubated at about 25°C.
25. A method according to any one of claims 16-24 wherein said embryos or ovaries are homogenised at about 4°C.
26. A method according to any one of claims 16-25 additionally comprising the step of adding glycerol to the recovered non-pelleted material to a concentration of about 10% by volume.
27. A method for the preparation of a mammalian cell extract comprising the steps of a) collecting cells by centrifugation; b) washing the cells; c) resuspending the cells; d)

homogenising the cells to produce a homogenate; e) centrifuging the homogenate and f) recovering non-pelleted material from the centrifuged homogenate.

28. A method for the *in vitro* translation of a ribonucleic acid template, said method comprising the steps of:

- 5 a) adding a ribonucleic acid template to a translation mix in the presence of an embryo extract produced by the method of any one of claims 16-26, an ovary cell extract produced by a method according to any one of claims 17-26, or a mammalian cell extract produced by the method of claim 27 to form a reaction mix,
- b) incubating the reaction mix for at least 90 minutes.

10 29. A method according to claim 28, wherein said reaction mix comprises spermidine, amino acids, creatine phosphate, creatine kinase, dithiothreitol (DTT), buffer,  $Mg(OAc)_2$ , KOAc, tRNA, embryo or cell extract, and RNA template.

30. A method according to claim 29, wherein said buffer is hypotonic buffer (10mM HEPES pH7.4, 5mM DTT).

5 31. A method according to either of claims 29 or 30, wherein said tRNA is calf liver tRNA.

32. A method according to any of claims 29-31, wherein the concentrations of the components of said reaction mix are as follows: spermidine, about 0.1mM; amino acids, about 60 $\mu$ M; DTT, 0-4mM; creatine phosphate, 17-23mM; creatine kinase, 0.08mg/ml; buffer, about 24mM;  $Mg(OAc)_2$ , 0-3mM; KOAc, 0-200mM; calf liver tRNA, 0-150 $\mu$ g/ml; embryo or cell extract, about 40% and RNA template, 1-3ng/ $\mu$ l.

0 33. A method according to claim 32, wherein the components of said reaction mix are as follows: spermidine, 0.1mM; amino acids, 60 $\mu$ M; DTT, 0-4mM; creatine phosphate, 17-23mM; creatine kinase, 0.08mg/ml; HEPES buffer, 24mM;  $Mg(OAc)_2$ , 0-3mM; KOAc, 60mM; calf liver tRNA, 100 $\mu$ g/ml; embryo or cell extract, 40% and RNA template, 3.2ng/ $\mu$ l.

5 34. A method according to any one of claims 28-33, wherein said reaction mix is prepared fresh.

35. A method according to any one of claims 29-34, wherein said creatine phosphate is prepared fresh or is newly thawed.
36. The method according to any one of claims 28-35 wherein said ribonucleic acid template is capped and contains a poly A tail of at least 30 adenine nucleotides in length.
37. A *Drosophila* embryo cell extract, ovary cell extract or mammalian cell extract produced by the method of any one of claims 16-27.
38. Use of embryo or cell extract according to claim 37 in a method of *in vitro* translation of a ribonucleic acid template.
39. A method according to any one of claims 1-15 wherein said cell extract is prepared according to the method of any one of claims 16 to 27.
40. A method of identifying a candidate molecule that increases or decreases the amount of a produced translation product, comprising:
- a) performing the method according to any one of claims 1-15, 28-36 or 39 wherein said conditions or reaction mix comprise the presence of one or more candidate molecules being screened for the ability to increase or decrease the amount of the encoded protein that is produced; and
  - b) identifying any one or more candidate molecules that increase or decrease the amount of the encoded protein that is produced relative to the amount of the encoded protein that is produced in the absence of the one or more candidate molecules.
41. A purified candidate molecule that is identified according to the method of claim 40.
42. A kit for *in vitro* translation of ribonucleic acid comprising the following ingredients: spermidine, amino acids, creatine phosphate, creatine kinase, optionally dithiothreitol, buffer,  $Mg(OAc)_2$ , KOAc, tRNA, *Drosophila* or mammalian cell extract according to claim 37, and RNA template.
43. A kit according to claim 42, wherein said buffer is HEPES buffer.

44. A kit according to either of claims 42 or 43, wherein said tRNA is calf liver tRNA.

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